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(57) Abstract

A composition and method for inhibiting the growth and metastasis of breast tumors, including a tumor immunogen derived from breast tumor cells treated with an antisense oligonucleotide complementary to a gene or mRNA for the receptor for insulin–like growth factor type 1.

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INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF-1R) ANTISENSE OLIGONUCLEOTIDE-TREATED BREAST CANCER CELLS COMPOSITION

Field of the Invention

This invention relates to a vaccine and to a therapeutic method effective for inhibiting the growth of breast tumors and inhibiting local or metastatic breast tumor reoccurrence. Specifically, the invention demonstrates that peripheral administration of breast tumor immunogens derived from breast tumor cells treated with an antisense oligonucleotide complementary to the gene or mRNA for IGF-1 receptor effectively inhibits the growth of breast tumor cells and prevents metastasis of tumor cells, e.g., to the brain.

Background Of the Invention

Breast cancer is the most common malignancy in females in North America, becoming clinically apparent in one out of nine women. The prevalence of breast cancer is high compared to its annual incidence in other countries (estimated at greater than four times that in the UK). Thus, a therapy for this disease would provide a significant benefit to a large number of individual patients as well as a relief of health care resources.

Current treatment for breast cancer is directed at non-specific elimination-resection, administration of agents toxic to growing cells, or inhibition of receptor ligands required for cell growth. Examples include radiation, cytotoxic chemotherapy (e.g., doxorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, mitomycin C and mitoxantrone) or hormonal manipulation to delete (e.g., ovarian ablation) or antagonize (e.g., tamoxifen, aromatase inhibitors) estrogen/progesterone stimulation of tumor growth. Because of their lack of tumor specificity, these therapies are poorly tolerated and become ineffective when the disease is widely metastatic.

These therapies have additional limitations. High doses of cytotoxic agents needed for therapeutic efficacy also destroy normal dividing immunological cells and gastrointestinal cells. Thus, administration of cytotoxic agents is limited by neutropenia, thrombocytopenia and malnutrition. Radiation and surgical

therapies are limited to relatively-localized disease. All strategies are limited by the degree of deformity and/or disability that patients are willing to tolerate for only a modest increase in survival. Thus, there is need for a therapy which specifically targets a patient's malignancy and does not reduce the quality of the patient's remaining life.

Breast cancers are difficult targets because they are heterogeneous in a variety of features, including, for example, presence and absence of estrogen and progesterone receptors, and presence and absence of amplified growth factor receptors. In addition, the tumor cells may have a variety of different mutations in somatic proto-oncogenes, such as c-*erb2*, c-*myc*, *int2*, *hst1*, *bcl1*, and *PRAD1*, or tumor suppresser genes, including *RB* and *TP53*. Further, a patient may have more then one malignant cell type in the same tumor.

A population of diversified targets however, is exactly what a host's immune system is designed to screen and selectively eliminate. Vaccine-based immunotherapy has been shown to be effective in treating animal models of other types of cancers. The diversity of targets in breast cancer plus effectiveness in other types of cancer suggests that development of a vaccine-based immunotherapy might be effective for the treatment of breast cancer.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to specified segments of a targeted gene or mRNA. The binding of an antisense oligonucleotide to DNA or RNA within a cell can inhibit translation or transcription in the cell, which can disrupt gene expression. Typically, antisense oligonucleotides are about 14 to about 25 nucleotides in length, since it is believed that at least about 14 bases are required to specifically target a unique mammalian gene sequence. The sequence of an antisense oligonucleotide is chosen to provide specificity for a particular mammalian gene or mRNA sequence.

Antisense oligonucleotides can be synthesized with either natural or synthetic bases and with a natural phosphate or modified phosphate or sugar backbone. For example, phosphothioate, phosphonate, and other backbone

modifications can significantly alter the biological half-life, and bioavailability of antisense oligonucleotides.

Advances in molecular biology and synthetic chemistry over the past two decades have stimulated interest in developing antisense oligonucleotides as therapeutic agents. It would be beneficial to develop an antisense oligonucleotide based therapy for breast cancer to provide targeted immunotherapy.

Insulin-like growth factor and its receptor

Growth factors and their receptors are examples of molecular switches whose activation transduces a signal to the cell nucleus that enables growth, transformation and protection from cell death. Insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) appear to be required for mitosis in many cell types. *In vitro*, most cells in culture are dependent on IGF-1 for growth, and many tumor lines secrete IGF-1 and express IGF-1R. IGF-1R is required for the entry of stimulated lymphocytes and HL-60 cells into S Phase. In the absence of proliferation, such as in senescent human fibroblasts, IGF-1 mRNA is not detectable by reverse transcriptase polymerase chain reaction. Once these senescent cells are transfected with a temperature sensitive SV40 T antigen gene, they regain the ability to express IGF-1 mRNA at permissive temperatures. IGF-1R is also associated with growth *in vivo*. Mice with homozygous null mutations for *igf-1r* gene die shortly after birth with a body weight 30% of wild type.

The expression of IGF-1R may be required for transformation *in vitro* and for tumor maintenance *in vivo*. When the gene encoding IGF-1R is disrupted in mouse embryo fibroblasts, transformation by either Ha-*ras*, SV40 tumor antigen or both, is prevented. The transformed phenotype is restored once cells are transfected with a plasmid expressing the IGF-1R RNA. Overexpression of the IGF-1R results in increased transformability of NIH 3T3 cells. Not only is oncogenesis associated with the induction of IGF genes, but preliminary data suggests that the gene product of the retinoblastoma tumor suppresser gene inhibits the expression of the IGF-1 gene.

The IGF-1 receptor (IGF-1R) is a membrane glycoprotein composed of two alpha (Mr 130,000) and two beta (Mr 98,000) subunits linked together by

disulfide bonds. The alpha subunit binds IGF-1 and IGF-2 with equal affinity. The β-subunit has an intracellular domain with tyrosine kinase activity which upon activation by either IGF-1 or IGF-2 autophosphorylates its own β-subunit and two major substrates, insulin receptor substrate 1 and Shc. Once activated, IGF-1R transmits a signal, transduced through *ras* and *raf*, to the nucleus. IGF-1 is known to stimulate the expression of approximately 30 genes expressed in 3T3 cells, encoding both cytoplasmic and nuclear proteins. Within one minute of IGF-1R ligand binding, a series of other cellular proteins as well as nuclear proteins including the 43 kD product of the *c-jun* protooncogene, are also phosphorylated *in vitro*.

The IGF-1R is required for the action of several growth factors. Antisense to IGF-1R blocks the EGF stimulated proliferation of 3T3 cells overexpressing EGFR. Neither PDGFR nor EGFR antisense inhibit IGF-1 stimulated growth in cells overexpressing IGF-1R. There is evidence that other protooncogenes, such as *c-myb*, induce expression of IGF-1 and IGF-1R. When *c-myb* is overexpressed in fibroblasts, the IGF-1 requirement for growth is lost because both IGF-1 and IGF-1R mRNA is induced. When *c-myb* expression is inhibited, there is a decrease in the IGF-1R mRNA. However, inhibition of IGF-1R expression has no effect on the *c-myb* mRNA levels.

IGF-1R antisense can inhibit the growth of cells whose growth depends on expression of IGF-1R. Cells that are exposed to IGF-1R antisense and cells that are transfected with a viral vector expressing IGF-1R antisense show diminished levels of IGF-1R protein. This results in growth inhibition in IGF-1R dependent cells like rat C6 glioblastoma cells, an IGF-1R dependent tumor cell line. Diminished tumorogenicity results from transfection of C6 glioblastoma cells with a viral vector expressing IGF-1R antisense. For example, no tumors developed in rats injected with C6 glioblastoma cells bearing a viral vector expressing IGF-1R antisense. Injection with these antisense-transfected cells can also protect rats from glioblastoma tumor formation due to subsequent injection of wild type C6 glioblastoma cells. No tumors appeared in 4 weeks after the injection of the wild-type cells. Injection of the antisense-transfected C6 glioblastoma cells also caused regression of previously established wild-type C6 glioblastoma tumors. In each of

these experiments the cells that exhibited diminished tumorogenicity or that affected tumor growth were transfected with a viral vector to express IGF-1R antisense.

Work, to date, studying IGF-1R antisense in cancer cells has been performed by transfecting cells with antisense DNA integrated in a viral vector. The consequences of inserting viral DNA into human gliomas are unknown. An estimated 23 human years of retroviral mediated gene transfer has been performed in humans without known side effects, although some of the viral vectors being used for gene therapy have the long-term potential for causing cancer. Side effects have been described in 3 monkeys at the NIH, who developed malignant T cell lymphoma after bone marrow transplant and gene transfer with a helper virus contaminated retrovirus. Aside from the potential side effects and risks of viral contamination, using viral vectors to transfect each tumor line with antisense is very labor intensive.

The practical use of antisense genetic therapy would be greatly enhanced by incorporation of antisense oligonucleotides into the breast, or other, tumor cells without the use of a retroviral vector. In light of the present shortcomings in breast cancer therapy, it would be advantageous to have a therapy that specifically inhibited the growth and metastasis of breast cancer cells without the side effects of present therapies. Antisense oligonucleotides present one avenue for such therapy, but present methods for treatment with antisense oligonucleotides require viral vectors and have not proven effective in inhibiting the growth and metastasis of breast cancer cells. Hence, there is a need for an antisense-oligonucleotide based therapy for breast cancer that eliminates use of viral vectors.

Summary of the Invention

The present invention includes a therapeutic composition useful for inhibiting the growth of breast cancer cells. The composition includes a tumor cell immunogen derived from breast cancer cells that have been treated with an IGF-1 receptor antisense oligonucleotide. Preferably, the treated breast cancer cells are inactivated, e.g., not viable.

The preferred immunogen is an inactivated cancer cell treated with IGF-1 receptor antisense oligonucleotide (AON). The immunogen can include

whole cells, cell membranes, lysates or extracts of cancer cells treated with IGF-1R antisense oligonucleotide.

The antisense oligonucleotide used to produce the immunogen is complementary to the gene or mRNA of the insulin-like growth factor type I receptor (IGF-1R). Preferably, the IGF-1R antisense oligonucleotide is about 14 to about 25 nucleotides in length. More preferably, the IGF-1R antisense oligonucleotide has a sequence complementary to the nucleotide sequence of the human IGF-1R precursor signal sequence, complementary to the nucleotide sequence at or near the initiation site, or complementary to sequence the nucleotide at or near a site for ribosome complex assembly.

More preferably, the sequence of the IGF-1 receptor antisense oligonucleotide contains the following sequence:

5' TCC TCC GGA GCC AGA CTT 3' (AON1) [SEQ ID NO:1], or

5' ACT CGT CGG CCA GAG CGA GAG 3' (AON2) [SEQ ID NO:2].

Advantageously, the IGF-1 receptor antisense oligonucleotide is modified, for example, by including synthetic bases or modifying the backbone, such as by substituting phosphothioates or phosphonates.

The invention also includes a method of preparing a therapeutic composition by treating breast cancer cells with an IGF-1 receptor antisense oligonucleotide.

Another embodiment of the invention is a tumor cell immunogen derived from a breast cancer cell treated with an IGF-1 receptor antisense oligonucleotide.

A further embodiment of the invention is an IGF-1 receptor antisense oligonucleotide having the sequence:

5' ACT CGT CGG CCA GAG CGA GAG 3' (AON2) [SEQ ID NO:2].

The invention also includes using the tumor cell immunogen in a method of immunizing a breast cancer patient; in a method of inhibiting growth of breast cancer cells; in a method of inhibiting metastasis of breast cancer cells, including the inhibition of breast cancer cell metastasis behind the blood-brain-barrier; and, in a method of treating breast cancer. Each of these methods includes administering to a patient a tumor immunogen produced by treating breast cancer

cells with an IGF-1 receptor antisense oligonucleotide and thereby inducing an anti-tumor response.

Brief Description of the Figures

FIG. 1 is a graph showing the effects of treatment with irradiated cells pretreated with IGF-1 receptor antisense oligonucleotide AON1 on tumor volume in rats with MAT3B breast tumors.

FIG. 2 is a graph showing the effect of treatment with irradiated cells pretreated with IGF-1 receptor antisense oligonucleotide AON1 on survival of rats with MAT3B breast tumors.

FIG. 3 is a graph showing the effect of treatment with irradiated cells pretreated with IGF-1 receptor antisense oligonucleotide AON2 on tumor volume in rats with MAT3B breast tumors.

FIG. 4 is a graph showing the effect of treatment with irradiated cells pretreated with IGF-1 receptor antisense oligonucleotide AON2 on survival of rats with MAT3B breast tumors.

FIG. 5 is a graph showing the effect of treatment with irradiated cells pretreated with IGF-1 receptor antisense oligonucleotides AON-1 or AON-2 on survival of rats with intracerebral tumors of MAT3B breast tumor cells.

FIG. 6 shows the nucleotide sequence of the cDNA clone of the IGF-1 receptor [SEQ ID NO:4] and the predicted amino acid sequence as reported by Ullrich et al., *EMBO J* 5:2503-2512 (1986). The α subunit includes the amino acids numbered 31-740, the putative precursor processing sequence is at amino acids 737-740, and the β subunit amino acids 741-1367. Amino acids 1-30 are a 30 residue signal sequence. Amino acids 936-959 are believed to form a transmembrane domain.

Detailed Description of the Preferred Embodiments

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences that are complementary to specified segments of a targeted gene or mRNA. In the present

invention, an IGF-1 receptor antisense oligonucleotide is complementary to a segment of the IGF-1 receptor gene, or to IGF-1 receptor mRNA.

The antisense oligonucleotides of the invention are preferably about 14-25 nucleotides in length. Most preferably, the oligonucleotides are about 15-20 nucleotides in length. It has been estimated that an antisense oligonucleotide containing at least 14 bases is required to specifically target a unique mammalian gene sequence, so longer oligonucleotides can provide greater specificity for targeting a specific sequence. However, small oligonucleotides are advantageous because they are typically more readily taken up by cells and can exhibit improved absorbtion and like properties. Thus, the most preferred size, 15-20 nucleotides, is chosen to balance target specificity and improved uptake by cells. The preferred oligonucleotide sequence can form Watson-Crick type base pairs along the entire target sequence, but less base pairing can be tolerated when advantageous cellular uptake, pharmacokinetics, or metabolism and adequate target recognition result from the altered sequence.

The particular sequence of the nucleotides provides the molecule with the specificity for targeting genetic material, such as DNA or mRNA. The antisense oligonucleotides of the invention have sequences that are complementary to the IGF-1 receptor gene or mRNA. Preferred oligonucleotides are those that are complementary to a sequence encoding the IGF-1 receptor precursor signal peptide, to sequences at or near the initiation site (AUG codon), or those sequences at or near a site for ribosome complex assembly, a site at which a ribosome binds to a polynucleotide. A sequence is near a site on an oligo- or polynucleotide if a complementary sequence would overlap with or interfere with the function of the site.

Most preferred antisense oligonucleotides of the invention are oligonucleotides AON1 [SEQ ID NO:1] and AON2 [SEQ ID NO:2] having the nucleotide sequences shown above. The antisense oligonucleotides can be produced by known, standard methods including chemical synthesis.

The antisense oligonucleotides of the invention can be chemically modified, such as by including synthetic bases or by modifications of the phosphate or sugar backbone. Such chemical modification can increase the stability and

biological half-life of these compounds by reducing sensitivity to degradation by exonucleases, such as 3'-exonucleases, and endonucleases. Preferred are modifications that affect the phosphate backbone such as phosphorothioate-, phophorodithioate-, methylphosphonate-, and phosphoramidite modified oligomers, as these are stable and typically resistant to degradation by nucleases. The biological half-life of a phosphorothioate antisense sequence, for example, has been determined to be approximately 48 hours. The ribose moiety of the sugar backbone can be modified as well. For example, 2'-methyl-ribonucleotides and alpha-anomer nucleotides can be incorporated into the antisense oligonucleotides. Production and properties of modified oligonucleotides is described in "*Oligonucleotides - Antisense Inhibitors of Gene Expression*", J.S. Cohen ed., CRC Press, Inc., Boca Raton, FL (1989).

The amount of antisense oligonucleotide required in the treatment of breast tumor cells is an amount effective to increase the activity of the breast tumor cells as tumor immunogen. Preferably, the antisense oligonucleotide is effective to produce an immunogen of potency such that a subject can be inoculated with an equivalent of about 2.5 X 10^9 treated cells per 75 kg person. In preparing the immunogen of the invention about $30x10^6$ cells are incubated in a solution of about 5 μM to about 80 μM antisense oligonucleotide, more preferably about 10 μM to about 40 μM antisense oligonucleotide. The antisense oligonucleotide can be added to cells in suspension or culture, in cell culture medium or another vehicle.

Mechanisms of Antisense Action

In the present invention, treatment of breast tumor cells with an IGF-1 receptor antisense oligonucleotide modifies the tumor cells to produce a breast tumor immunogen. Such a modified breast tumor cell is a preferred tumor immunogen for use to inhibit the growth of breast cancer cells.

Although not limiting to the present invention, there are several mechanisms that can account for production of such an immunogen. For example, treatment with the antisense oligonucleotide could result in a change in phenotype of the treated cell, so the cell becomes a tumor immunogen. Alternatively, gene expression could be altered with resulting increased expression or display of an

antigen or immunogen to breast tumor on the treated cells. It is believed that binding of an antisense oligonucleotide to DNA or RNA within a cell can inhibit translation and transcription, which can disrupt gene expression.

Several theories have been advanced to explain the general action of antisense oligonucleotides in a variety of systems. Proposed mechanisms of action for antisense oligonucleotides can be classified as passive, reactive, and activating. Passive blocking of function occurs by steric hindrance whereby an mRNA molecule cannot effectively interact with ribosomes, or is unable to pass from the nucleus to the cytoplasm. Reactive processes occur where antisense oligonucleotides bind directly with a target sequence and either block its action or cause its cleavage.

For example, binding of antisense oligonucleotides to target DNA can interrupt gene transcription, or if the antisense oligonucleotide binds to mRNA, translation will be disrupted. Consequently, the synthesis of a particular protein product can be inhibited. In cell-free translation systems, the best mRNA target sites are considered the 5' end (at or near the initiation AUG codon) and at or near the sites for ribosome complex assembly. Cleavage is advantageous because it destroys the target DNA or RNA. In addition, when a target DNA or RNA is cleaved, the antisense oligonucleotide will be released intact to recycle and bind to other target sequences. The activating mechanisms of RNase H result in the digestion of the target mRNA irrespective of the point of antisense attachment.

Modification of the antisense oligonucleotide can have advantageous effects on the mechanism or action of the antisense oligonucleotide. For example, phosphodiester antisense oligonucleotides hybridize very efficiently to complementary RNA sequences and thereby block translation, but they also can recruit the enzyme RNase H which cleaves the RNA component of the RNA/DNA duplex. Methylphosphonate antisense oligonucleotides are readily taken up by cells, have low toxicity and high stability, however, they hybridize poorly and may not induce the activity of RNase. Phophorothiaoate antisense oligonucleotides, in contrast, hybridize efficiently and induce RNase in a concentration-dependent fashion. Based on these theories of action, phosphorothioate antisense oligonucleotides with resistance to endonuclease activity can be advantageous in the immunogens and methods of the invention.

Treated Tumor Cells

Tumor immunogen of the present invention is preferably whole tumor or cancer cells that have been treated with an antisense oligonucleotide complementary to the IGF-I receptor gene or mRNA. Immunogen, as used herein, refers to a substance that can induce an immune response, including either a humoral and/or cellular immune response. The tumor immunogen may also be derived from treated tumor cells by subjecting them to processes such as altering growth media, washing, purification, isolation, freezing, inactivation, lysis, extraction, and the like.

Treatment can include a single dose or multiple doses of antisense oligonucleotide. Preferably, the tumor cells are treated with two doses of antisense oligonucleotide. Typically, when treatment includes two doses of oligonucleotide, one dose of antisense is added at the beginning of the incubation and a second is added about half way through the incubation. Preferably, for treatment, the cells are incubated with antisense oligonucleotide for about 23 hours, then maintained in culture for about one to two hours prior to use as a vaccine or immunogen, or before further processing. Treatment of cells is preferably under culture conditions favorable for the tumor cell type, generally at a temperature of 37°C and in a nutrient medium such as RPMI cell culture medium.

After treatment with antisense oligonucleotide, the treated cells are inactivated, preferably by irradiation. Treated, inactivated cells are preserved by methods, such as quick freezing at -80°C, that maintain the immunogenicity of the stored cells. This process yields tumor immunogen derived from treated breast cancer cells. The conditions of culture and incubation with the antisense oligonucleotide can be chosen to provide for effective production of tumor immunogen.

The tumor immunogen is preferably prepared from a specific patient's tumor, e.g. from biopsy tissue or from explants of a removed tumor, or from cell culture of the patient's tumor cells. A patient's breast tumor cells can be obtained by standard biopsy methods. Cells from excised tumor tissue can be used directly, or alternatively, cells from the excised tumor can be cultured and expanded under standard culture conditions to produce increased numbers of cells. Tumor

cells from a patient can also be used to establish a permanent tumor cell line that can then be treated to make the immunogen of the invention, the immunogen or vaccine of the invention, or used in the methods of the invention.

Tumor cells are preferably inactivated, e.g., by methods known in the field, the most common method being irradiation as described in the examples below. Other known inactivation methods include oxygen deprivation, use of plant and animal toxins, and chemotherapeutic agents. In an alternative embodiment, lysed tumor cells may be used as the tumor immunogen, as well as cell membranes and specific tumor cell protein immunogens; however, inactivated whole tumor cells are preferred.

For administration, the tumor immunogen is suspended in an aqueous medium such as phosphate buffered saline. The amount of tumor immunogen administered is that sufficient to induce an immune response. In rat studies, administration of 5×10^6 irradiated tumor cells in 300μL phosphate buffered saline was efficient in preventing tumor growth. Thus, the expected useful human dose of irradiated tumor cells for a subcutaneous injection is about 2.5×10^9 cells per 75 kg person. Administration of other types of tumor immunogens, e.g. cell membrane or purified tumor cell immunogens, are administered to deliver a like amount of immunogen.

The therapeutic composition of the invention includes tumor cell immunogen in a form suitable for administration to a patient. For example, the composition can include adjuvants, cofactors and pharmaceutically acceptable carriers, vehicles, or buffers, and the like to formulate the composition for administration to a patient. The therapeutic composition can include one or more forms of the tumor cell immunogen in quantities effective to stimulate an immune response in the patient. The therapeutic composition of the invention can formulated and administered as a vaccine. Vaccine, as used herein, refers to a therapeutic composition formulated for administration to prevent, ameliorate, or treat a disease, such as cancer.

Methods of Administering

For the vaccine, immunogen and methods of the invention, administration of breast tumor immunogen for inhibiting the growth of breast cancer cells and the treatment of breast tumors is to a peripheral site, preferably by subcutaneous injection. Preferred peripheral sites for administration include the upper arm, thigh, and trunk areas of the body.

Timing of tumor immunogen administration is as needed to produce immune reactivity, and can be monitored by assessing change in tumor size (e.g., by MRI), immune response (e.g., by delayed type hypersensitivity skin test), and by measuring interferon-gamma secretion by the patient's TH-1 cells in response to tumor immunogen. Treatment is preferably continued until immune response is detected and/or tumor ablation is achieved.

Patient Populations

Patients at risk of or suffering from all types of breast tumors are treated by the method of the invention. A successful peripheral administration of tumor immunogen for the treatment of breast tumor is surprising, given the distant location of the tumors. The claimed method of treating with breast cancer tumor immunogen is particularly useful in preventing tumor recurrence, for example, after tumor reduction techniques, such as surgical debulking removal, irradiation, and/or chemotherapy.

Treating breast cancer tumors can also result in inhibition of growth of breast tumor cells, tumor regression, delay in or slowing of tumor growth, reduction in tumor mass, and other beneficial effects on tumor progression. Inhibiting growth of breast tumor cells includes slowing division or killing the cells. Inhibited cell growth leads to slower tumor growth, which depends on growth of individual cells. Treating or preventing metastasis includes stopping, slowing, or delaying the spread of the breast cancer tumor cells to other tissues or organs in the patient. Preventing breast cancer in a patient refers to treatment of patients at risk of breast cancer to delay or prevent the onset of breast cancer. Prevention can be measured as a reduction of incidence of breast cancer in a population at risk of breast cancer. Alternatively, preventing cancer in breast cancer survivors includes

inhibiting recurrence or regrowth of breast cancer. Administration of the therapeutic composition of the invention effectively inhibits regrowth of tumor, e.g. from residual tumor cells.

Examples

The invention will be further described by reference to the following detailed examples, which are exemplary in nature and not intended to limit the scope of the invention.

Example 1
Vaccination with Breast Tumor Cells Treated with IGF-1 Receptor (AON1) Antisense Oligonucleotide

Two IGF-1 receptor antisense oligonucleotides have been used to produce tumor cell immunogens for the inhibition of breast cancer growth. The first sequence used was a known antisense sequence, AON1 [SEQ ID NO:1], complementary to the nucleotide sequence of codons -29 to -24 (nucleotides -87 to -70) of the human IGF-1R precursor signal sequence. The sequence of AON1 is:

5' TCC TCC GGA GCC AGA CTT 3'

The second antisense oligonucleotide tested is AON2, [SEQ ID NO:2] described more fully below in Example 2, and having the following sequence:

5' ACT CGT CGG CCA GAG CGA GAG 3'

Vaccine Production

To provide tumor immunogens for immune stimulation, MAT3B cells were grown at 37°C until reaching 80 - 90 % confluence in 175 mm^3 flasks of DMEM media supplemented with glycine, penicillin, streptomycin and 10% fetal calf serum. These cells were then collected, centrifuged at 1000 g x 5 minutes, washed twice in ice cold PBS, centrifuged and resuspended in each of three 175 mm^3 flasks in 10 mls of serum free DMEM media supplemented with 12.5 μM IGF-1 (Upstate Biotechnology), 0.10% BSA and 10 μM $FeSO_4$.

These three flasks were used to make cells for an antisense oligonucleotide treatment, a treatment with nonsense oligonucleotide, and a vehicle control treatment. Antisense treated cells were incubated with 12 μM IGF-1R AON (NBI, Plymouth, MN) in the first flask. Nonsense treated cells were incubated with 12 μM IGF-1R NON in the second flask. For the vehicle control treatment, an volume of oligonucleotide buffer (PBS) equivalent to the volume used in the other flasks was added to a third flask. Cells under treatment were incubated for 24 hours and redosed with 6 μM IGF-1R AON, 6 μM IGF-1R NON, or oligonucleotide buffer (PBS) during the last hour of incubation prior to harvest.

The Mat3B cells were then harvested, counted with a hemocytometer, resuspended in PBS at a concentration of 10^6 per 300 μl PBS, and irradiated with 6000 rads ^{137}Cs. Irradiated cells were then quick frozen in liquid nitrogen and stored at -70 °C until use (storage time varied between 0 and 48 hours).

Animal Vaccination

Female Fischer 344 rats, 150-160 gm and syngeneic for Mat3B cells, were obtained from Harlan (Indianapolis, IN) and cared for according to the University of Minnesota guidelines. Animals were either unvaccinated controls or vaccinated with Mat3B cells treated with IGF-1R AON, IGF-1R NON, or oligonucleotide buffer (PBS) as described under vaccine production. One of two vaccination protocols were used. For AON1 treated breast tumor cells, animals were vaccinated with subcutaneous hind limb injections of treated cells at 2 months and 1 month prior to tumor challenge. For AON2 or nonsense oligonucleotide treated breast tumor cells, animals were vaccinated with subcutaneous hind limb injections of treated cells or at 6 weeks, 4 weeks and 2 weeks prior to tumor challenge. Animals were vaccinated with 5 X 10^6 cells in 300 μl of PBS.

Peripheral Tumor Inoculation and Evaluation

To establish a model in which tumor growth or regression could be easily quantitated on a daily basis peripheral breast cancers were then established in the opposite hind flank of the vaccinated, or unvaccinated syngeneic rodents described above. Peripheral tumors were established by injecting 10^6 unmodified

log phase Mat3B cells that had been harvested, washed and resuspended in 300 µl PBS.

Tumor lengths and widths were then measured daily by an observer who was blinded to the vaccination group. Tumor volumes were approximated using the equation $[(\text{width})^2 \times \text{length}] / 2$, and averages ± SEM were calculated daily for each group. Daily average tumor volumes from each group were compared over the course of the experiment using ANOVA and *post hoc* TUKEY with the SAS statistical analysis program. Groups averages were not compared after one or more animals in the group died or after all animals in the vaccination group recovered from their tumors.

Those rats which were resistant to or recovered from peripheral breast cancer were then monitored without further treatment for 2 months (AON2 or NON vaccination) to 7 months (AON1 vaccination). The tumor free rats were then rechallenged with peripheral breast cancer inoculations as above.

Results

Figure 1 shows the effect of vaccination with AON1-treated tumor cells on breast tumor volume. Both unvaccinated naive animals and animals tested with phosphate buffered saline (PBS) treated cells exhibited rapid tumor growth. In contrast, animals immunized with AON1-treated tumor cells exhibited a significant delay in tumor growth. In this latter group two animals displayed a regression of tumor volume and a third animal never developed a palpable tumor.

The survival of animals vaccinated with irradiated AON1 treated MAT3B tumor cells is shown in Figure 2. Naive control animals all died within 25 days of breast tumor implantation. Animals vaccinated with NaCl treated cells all died within 38 days. In contrast, 40% of animals with IGF-1 receptor antisense (AON1) vaccinations survived beyond 250 days post tumor implantation and continue to survive.

These studies demonstrate IGF-1 Receptor antisense oligonucleotide (AON1) when incubated with breast tumor cells produces a vaccine that prevents breast tumor cell growth in vaccinated animals.

Example 2
Vaccination with Breast Tumor Cells Treated with IGF-1 Receptor (AON2) Antisense Oligonucleotide

A second antisense sequence IGF-1R AON2 was designed using a selected region of the IGF-1R gene sequence having the following sequence:

5′ ACT CGT CGG CCA GAG CGA GAG 3′ [SEQ ID NO:2]

A nonsense sequence used as a control having the following sequence:

5′ TGG CAC CGT ACC AGG AGG CAG 3′ [SEQ ID NO:3]

The IGF-1R AON2 sequence is complementary to a region of the IGF-1R gene that is homologous between humans, mice, and rats:

...GCG**CTCTCGCTCTGGCCGACGAGT**GGAGAAATCTGC...Human
...GCG**CTCTCGCTCTGGCCGACGAGT**GGAGAAATTTGT...Mouse
...GCG**CTCTCGCTCTGGCCGACGAGT**GGAGAAATTTGT...Rat

Breast tumor cell vaccines were prepared as described in Example 1 by incubating MAT3B cells with the IGF-1 receptor antisense oligonucleotide AON2. Control vaccines were prepared by incubating MAT3B cells with the nonsense oligonucleotide (NON) and with PBS treated cells were irradiated, as described for Example 1. Syngeneic animals were immunized with the treated irradiated cells at 6, 4, and 2 weeks prior to breast tumor cell implantation, as described in Example 1. Peripheral tumors were established and evaluated as described in Example 1.

Results

The effects of this vaccine, AON2-treated tumor cells, on breast tumor growth is shown in Figure 3. Unvaccinated control animals exhibited rapid breast tumor growth as did animals vaccinated with PBS-incubated cells. Animals vaccinated with tumor cells treated with the nonsense oligonucleotide, IGF-1R NON, displayed a slightly slower growth rate of the tumor. The slowest tumor growth rate was exhibited by animals vaccinated with tumor cells treated with the

novel antisense oligonucleotide, AON2. One animal in this latter group had no palpable tumor, and one other animal exhibited a regression of its tumor.

The survival of animals vaccinated with AON2-treated cells is shown in Figure 4. Untreated control animals died within 19 days after breast tumor implantation. Animals vaccinated with PBS-treated cells or cells treated with nonsense oligonucleotide all succumbed to their cancer by day 28. In contrast, 33% of the animals vaccinated with AON2-treated cells have survived beyond 125 days and continue to survive.

These studies demonstrate IGF-1 Receptor antisense oligonucleotide (AON2) incubated with breast tumor cells produces a vaccine that inhibits breast tumor cell growth in immunized animals.

Example 3

The Effect the AON1 Vaccine on Breast Tumor Metastasis to the Brain

The therapeutic effects of animal vaccination with IGF-1 receptor antisense oligonucleotide treated cells were also examined in a model of breast tumor metastasis to the brain. Fischer 344 rats were vaccinated with either IGF-1R AON1- or AON2-treated irradiated MAT3B cells prepared and vaccinated as described above for Example 1.

Central Tumor Inoculation and Evaluation

In the model for breast cancer metastasis, 10^6 unmodified, log phase Mat3B cells were suspended in 10 µl PBS and injected into the striatum of vaccinated or unvaccinated syngeneic rats. To assess the length of protection provided by the vaccine, these animals were vaccinated 3 months (group 2) to 8 months (group 1) prior to intracerebral challenge.

Injections were made at the rate of 1 µl per minute with a 5 minute pause prior to removal of the Hamilton syringe. Animals were monitored for survival. In long term survivors, the presence or absence of tumors was assessed with MRI of animals anesthetized with ketamine/xylozine.

Results

All control animals (n = 10) died by day 8 after the intracerebral injections because of tumor growth within the brain (see Table 1 and FIG. 5). In contrast, 75% of the rats previously immunized with either IGF-1R AON1 or AON2-treated tumor cells survived 90 days beyond the intracerebral injection and continue to survive (FIG. 5). There was no significant difference between the animals treated with AON-1 or AON-2, and the data for these two treatments were pooled in the results reported in Table 1 and FIG. 5.

Table 1. Survival of rats with intracerebral tumors of breast carcinoma cells

Non-immunized control rats	(0/10)	0%
IGF-1R AON immunized rats	(3/4)	75%

MRI of immunized and non-immunized rats demonstrated the prevention of metastatic MAT3B breast tumor growth in the brain and the regression of tumors in vaccinated animals.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Regents of the University of Minnesota

(ii) TITLE OF THE INVENTION: INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF-1R) ANTISENSE OLIGONUCLEOTIDE CELLS COMPOSITION

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Unknown
(B) FILING DATE: 14-NOV-1997
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: 08/755,558
(B) FILING DATE: 22-NOV-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCCTCCGGAG CCAGACTT 18

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACTCGTCGGC CAGAGCGAGA G 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGCACCGTA CCAGGAGGCA G 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4989 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 46...4149
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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                                                  Met Lys Ser Gly
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TCC GGA GGA GGG TCC CCG ACC TCG CTG TGG GGG CTC CTG TTT CTC TCC      105
Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp Gly Leu Leu Phe Leu Ser
 5                  10                  15                  20

GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA ATC TGC GGG CCA GGC      153
Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile Cys Gly Pro Gly
                25                  30                  35

ATC GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAG AAC TGC      201
Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn Cys
            40                  45                  50

ACG GTG ATC GAG GGC TAC CTC CAC ATC CTG CTC ATC TCC AAG GCC GAG      249
Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile Ser Lys Ala Glu
        55                  60                  65

GAC TAC CGC AGC TAC CGC TTC CCC AAG CTC ACG GTC ATT ACC GAG TAC      297
Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu Tyr
    70                  75                  80

TTG CTG CTG TTC CGA GTG GCT GGC CTC GAG AGC CTC GGA GAC CTC TTC      345
Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu Phe
85                  90                  95                  100

CCC AAC CTC ACG GTC ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC GCC      393
Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr Ala
                105                 110                 115

CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC AAC      441
Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr Asn
            120                 125                 130

CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT GAC      489
Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala Asp
        135                 140                 145

CTC TGT TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG GTG      537
Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala Val
    150                 155                 160

TCC AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG GAC      585
Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly Asp
165                 170                 175                 180

CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC ACC      633
Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr Thr
                185                 190                 195
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ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC TGC CAG      681
Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys Gln
            200                 205                 210

AAA ATG TGC CCA AGC ACG TGT GGG AAG CGG GCG TGC ACC GAG AAC AAT      729
Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr Glu Asn Asn
        215                 220                 225

GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC GCG CCT GAC AAC      777
Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser Ala Pro Asp Asn
    230                 235                 240

GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC TAT GCC GGT GTC TGT      825
Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr Ala Gly Val Cys
245                 250                 255                 260

GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG TTT GAG GGC TGG CGC TGT      873
Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu Gly Trp Arg Cys
                265                 270                 275

GTG GAC CGT GAC TTC TGC GCC AAC ATC CTC AGC GCC GAG AGC AGC GAC      921
Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala Glu Ser Ser Asp
            280                 285                 290

TCC GAG GGG TTT GTG ATC CAC GAC GGC GAG TGC ATG CAG GAG TGC CCC      969
Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met Gln Glu Cys Pro
        295                 300                 305

TCG GGC TTC ATC CGC AAC GGC AGC CAG AGC ATG TAC TGC ATC CCT TGT     1017
Ser Gly Phe Ile Arg Asn Gly Ser Gln Ser Met Tyr Cys Ile Pro Cys
    310                 315                 320

GAA GGT CCT TGC CCG AAG GTC TGT GAG GAA GAA AAG AAA ACA AAG ACC     1065
Glu Gly Pro Cys Pro Lys Val Cys Glu Glu Glu Lys Lys Thr Lys Thr
325                 330                 335                 340

ATT GAT TCT GTT ACT TCT GCT CAG ATG CTC CAA GGA TGC ACC ATC TTC     1113
Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly Cys Thr Ile Phe
                345                 350                 355

AAG GGC AAT TTG CTC ATT AAC ATC CGA CGG GGG AAT AAC ATT GCT TCA     1161
Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile Ala Ser
            360                 365                 370

GAG CTG GAG AAC TTC ATG GGG CTC ATC GAG GTG GTG ACG GGC TAC GTG     1209
Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly Tyr Val
        375                 380                 385
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AAG ATC CGC CAT TCT CAT GCC TTG GTC TCC TTG TCC TTC CTA AAA AAC     1257
Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu Lys Asn
    390                 395                 400

CTT CGC CTC ATC CTA GGA GAG GAG CAG CTA GAA GGG AAT TAC TCC TTC     1305
Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr Ser Phe
405                 410                 415                 420

TAC GTC CTC GAC AAC CAG AAC TTG CAG CAA CTG TGG GAC TGG GAC CAC     1353
Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp Asp His
                425                 430                 435

CGC AAC CTG ACC ATC AAA GCA GGG AAA ATG TAC TTT GCT TTC AAT CCC     1401
Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe Ala Phe Asn Pro
            440                 445                 450

AAA TTA TGT GTT TCC GAA ATT TAC CGC ATG GAG GAA GTG ACG GGG ACT     1449
Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val Thr Gly Thr
        455                 460                 465

AAA GGG CGC CAA AGC AAA GGG GAC ATA AAC ACC AGG AAC AAC GGG GAG     1497
Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg Asn Asn Gly Glu
    470                 475                 480

AGA GCC TCC TGT GAA AGT GAC GTC CTG CAT TTC ACC TCC ACC ACC ACG     1545
Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe Thr Ser Thr Thr Thr
485                 490                 495                 500

TCG AAG AAT CGC ATC ATC ATA ACC TGG CAC CGG TAC CGG CCC CCT GAC     1593
Ser Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr Arg Pro Pro Asp
                505                 510                 515

TAC AGG GAT CTC ATC AGC TTC ACC GTT TAC TAC AAG GAA GCA CCC TTT     1641
Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys Glu Ala Pro Phe
            520                 525                 530

AAG AAT GTC ACA GAG TAT GAT GGG CAG GAT GCC TGC GGC TCC AAC AGC     1689
Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala Cys Gly Ser Asn Ser
        535                 540                 545

TGG AAC ATG GTG GAC GTG GAC CTC CCG CCC AAC AAG GAC GTG GAG CCC     1737
Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys Asp Val Glu Pro
    550                 555                 560

GGC ATC TTA CTA CAT GGG CTG AAG CCC TGG ACT CAG TAC GCC GTT TAC     1785
Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln Tyr Ala Val Tyr
565                 570                 575                 580

GTC AAG GCT GTG ACC CTC ACC ATG GTG GAG AAC GAC CAT ATC CGT GGG     1833
Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile Arg Gly
                585                 590                 595

GCC AAG AGT GAG ATC TTG TAC ATT CGC ACC AAT GCT TCA GTT CCT TCC     1881
Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val Pro Ser
            600                 605                 610
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ATT CCC TTG GAC GTT CTT TCA GCA TCG AAC TCC TCT TCT CAG TTA ATC     1929
Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln Leu Ile
        615                 620                 625

GTG AAG TGG AAC CCT CCC TCT CTG CCC AAC GGC AAC CTG AGT TAC TAC     1977
Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn Leu Ser Tyr Tyr
    630                 635                 640

ATT GTG CGC TGG CAG CGG CAG CCT CAG GAC GGC TAC CTT TAC CGG CAC     2025
Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Tyr Arg His
645                 650                 655                 660

AAT TAC TGC TCC AAA GAC AAA ATC CCC ATC AGG AAG TAT GCC GAC GGC     2073
Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala Asp Gly
                665                 670                 675

ACC ATC GAC ATT GAG GAG GTC ACA GAG AAC CCC AAG ACT GAG GTG TGT     2121
Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys Thr Glu Val Cys
            680                 685                 690

GGT GGG GAG AAA GGG CCT TGC TGC GCC TGC CCC AAA ACT GAA GCC GAG     2169
Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu Ala Glu
        695                 700                 705

AAG CAG GCC GAG AAG GAG GAG GCT GAA TAC CGC AAA GTC TTT GAG AAT     2217
Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe Glu Asn
    710                 715                 720

TTC CTG CAC AAC TCC ATC TTC GTG CCC AGA CCT GAA AGG AAG CGG AGA     2265
Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Lys Arg Arg
725                 730                 735                 740

GAT GTC ATG CAA GTG GCC AAC ACC ACC ATG TCC AGC CGA AGC AGG AAC     2313
Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser Arg Asn
                745                 750                 755

ACC ACG GCC GCA GAC ACC TAC AAC ATC ACC GAC CCG GAA GAG CTG GAG     2361
Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu Leu Glu
            760                 765                 770

ACA GAG TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC ACG GAG AGA ACT     2409
Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Thr Glu Arg Thr
        775                 780                 785

GTC ATT TCT AAC CTT CGG CCT TTC ACA TTG TAC CGC ATC GAT ATC CAC     2457
Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Arg Ile Asp Ile His
    790                 795                 800

AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC AAC TTC     2505
Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser Asn Phe
805                 810                 815                 820

GTC TTT GCA AGG ACT ATG CCC GCA GAA GGA GCA GAT GAC ATT CCT GGG     2553
Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile Pro Gly
                825                 830                 835
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CCA GTG ACC TGG GAG CCA AGG CCT GAA AAC TCC ATC TTT TTA AAG TGG     2601
Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu Lys Trp
            840                 845                 850

CCG GAA CCT GAG AAT CCC AAT GGA TTG ATT CTA ATG TAT GAA ATA AAA     2649
Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met Tyr Glu Ile Lys
        855                 860                 865

TAC GGA TCA CAA GTT GAG GAT CAG CGA GAA TGT GTG TCC AGA CAG GAA     2697
Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val Ser Arg Gln Glu
    870                 875                 880

TAC AGG AAG TAT GGA GGG GCC AAG CTA AAC CGG CTA AAC CCG GGG AAC     2745
Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu Asn Pro Gly Asn
885                 890                 895                 900

TAC ACA GCC CGG ATT CAG GCC ACA TCT CTC TCT GGG AAT GGG TCG TGG     2793
Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly Asn Gly Ser Trp
                905                 910                 915

ACA GAT CCT GTG TTC TTC TAT GTC CAG GCC AAA ACA GGA TAT GAA AAC     2841
Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr Gly Tyr Glu Asn
            920                 925                 930

TTC ATC CAT CTG ATC ATC GCT CTG CCC GTC GCT GTC CTG TTG ATC GTG     2889
Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val Leu Leu Ile Val
        935                 940                 945

GGA GGG TTG GTG ATT ATG CTG TAC GTC TTC CAT AGA AAG AGA AAT AAC     2937
Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg Lys Arg Asn Asn
    950                 955                 960

AGC AGG CTG GGG AAT GGA GTG CTG TAT GCC TCT GTG AAC CCG GAG TAC     2985
Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val Asn Pro Glu Tyr
965                 970                 975                 980

TTC AGC GCT GCT GAT GTG TAC GTT CCT GAT GAG TGG GAG GTG GCT CGG     3033
Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp Glu Val Ala Arg
                985                 990                 995

GAG AAG ATC ACC ATG AGC CGG GAA CTT GGG CAG GGG TCG TTT GGG ATG     3081
Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly Ser Phe Gly Met
            1000                1005                1010

GTC TAT GAA GGA GTT GCC AAG GGT GTG GTG AAA GAT GAA CCT GAA ACC     3129
Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp Glu Pro Glu Thr
        1015                1020                1025

AGA GTG GCC ATT AAA ACA GTG AAC GAG GCC GCA AGC ATG CGT GAG AGG     3177
Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser Met Arg Glu Arg
    1030                1035                1040

ATT GAG TTT CTC AAC GAA GCT TCT GTG ATG AAG GAG TTC AAT TGT CAC     3225
Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn Cys His
1045                1050                1055                1060
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CAT GTG GTG CGA TTG CTG GGT GTG GTG TCC CAA GGC CAG CCA ACA CTG     3273
His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly Gln Pro Thr Leu
                1065                1070                1075

GTC ATC ATG GAA CTG ATG ACA CGG GGC GAT CTC AAA AGT TAT CTC CGG     3321
Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys Ser Tyr Leu Arg
            1080                1085                1090

TCT CTG AGG CCA GAA ATG GAG AAT AAT CCA GTC CTA GCA CCT CCA AGC     3369
Ser Leu Arg Pro Glu Met Glu Asn Asn Pro Val Leu Ala Pro Pro Ser
        1095                1100                1105

CTG AGC AAG ATG ATT CAG ATG GCC GGA GAG ATT GCA GAC GGC ATG GCA     3417
Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala
    1110                1115                1120

TAC CTC AAC GCC AAT AAG TTC GTC CAC AGA GAC CTT GCT GCC CGG AAT     3465
Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn
1125                1130                1135                1140

TGC ATG GTA GCC GAA GAT TTC ACA GTC AAA ATC GGA GAT TTT GGT ATG     3513
Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met
                1145                1150                1155

ACG CGA GAT ATC TAT GAG ACA GAC TAT TAC CGG AAA GGA GGC AAA GGG     3561
Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly
            1160                1165                1170

CTG CTG CCC GTG CGC TGG ATG TCT CCT GAG TCC CTC AAG GAT GGA GTC     3609
Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp Gly Val
        1175                1180                1185

TTC ACC ACT TAC TCG GAC GTC TGG TCC TTC GGG GTC GTC CTC TGG GAG     3657
Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp Glu
    1190                1195                1200

ATC GCC ACA CTG GCC GAG CAG CCC TAC CAG GGC TTG TCC AAC GAG CAA     3705
Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn Glu Gln
1205                1210                1215                1220

GTC CTT CGC TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCA GAC AAC     3753
Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro Asp Asn
                1225                1230                1235

TGT CCT GAC ATG CTG TTT GAA CTG ATG CGC ATG TGC TGG CAG TAT AAC     3801
Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln Tyr Asn
            1240                1245                1250

CCC AAG ATG AGG CCT TCC TTC CTG GAG ATC ATC AGC AGC ATC AAA GAG     3849
Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser Ser Ile Lys Glu
        1255                1260                1265

GAG ATG GAG CCT GGC TTC CGG GAG GTC TCC TTC TAC TAC AGC GAG GAG     3897
Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr Tyr Ser Glu Glu
    1270                1275                1280
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AAC AAG CTG CCC GAG CCG GAG GAG CTG GAC CTG GAG CCA GAG AAC ATG     3945
Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu Pro Glu Asn Met
1285                1290                1295                1300

GAG AGC GTC CCC CTG GAC CCC TCG GCC TCC TCG TCC TCC CTG CCA CTG     3993
Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser Ser Leu Pro Leu
                1305                1310                1315

CCC GAC AGA CAC TCA GGA CAC AAG GCC GAG AAC GGC CCC GGC CCT GGG     4041
Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly Pro Gly Pro Gly
            1320                1325                1330

GTG CTG GTC CTC CGC GCC AGC TTC GAC GAG AGA CAG CCT TAC GCC CAC     4089
Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln Pro Tyr Ala His
        1335                1340                1345

ATG AAC GGG GGC CGC AAG AAC GAG CGG GCC TTG CCG CTG CCC CAG TCT     4137
Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro Leu Pro Gln Ser
    1350                1355                1360

TCG ACC TGC TGA TCCTTGGATC CTGAATCTGT GCAAACAGTA ACGTGTGCGC ACGCGC  4195
Ser Thr Cys
1365

AGCGGGGTGG GGGGGGAGAG AGAGTTTTAA CAATCCATTC ACAAGCCTCC TGTACCTCAG   4255
TGGATCTTCA GTTCTGCCCT TGCTGCCCGC GGGAGACAGC TTCTCTGCAG TAAAACACAT   4315
TTGGGATGTT CCTTTTTTCA ATATGCAAGC AGCTTTTTAT TCCCTGCCCA AACCCTTAAC   4375
TGACATGGGC CTTTAAGAAC CTTAATGACA ACACTTAATA GCAACAGAGC ACTTGAGAAC   4435
CAGTCTCCTC ACTCTGTCCC TGTCCTTCCC TGTTCTCCCT TTCTCTCTCC TCTCTGCTTC   4495
ATAACGGAAA AATAATTGCC ACAAGTCCAG CTGGGAAGCC CTTTTTATCA GTTTGAGGAA   4555
GTGGCTGTCC CTGTGGCCCC ATCCAACCAC TGTACACACC CGCCTGACAC CGTGGGTCAT   4615
TACAAAAAAA CACGTGGAGA TGGAAATTTT TACCTTTATC TTTCACCTTT CTAGGGACAT   4675
GAAATTTACA AAGGGCCATC GTTCATCCAA GGCTGTTACC ATTTTAACGC TGCCTAATTT   4735
TGCCAAAATC CTGAACTTTC TCCCTCATCG GCCCGGCGCT GATTCCTCGT GTCCGGAGGC   4795
ATGGGTGAGC ATGGCAGCTG GTTGCTCCAT TTGAGAGACA CGCTGGCGAC ACACTCCGTC   4855
CATCCGACTG CCCCTGCTGT GCTGCTCAAG GCCACAGGCA CACAGGTCTC ATTGCTTCTG   4915
ACTAGATTAT TATTTGGGGG AACTGGACAC AATAGGTCTT TCTCTCAGTG AAGGTGGGGA   4975
GAAGCTGAAC CGGC                                                     4989
```

We Claim:

1. A therapeutic composition for inhibiting the growth of breast cancer cells, comprising:

an immunogen derived from breast cancer cells treated with an IGF-1 receptor antisense oligonucleotide.

2. The composition of claim 1, wherein the immunogen comprises IGF-1 receptor antisense oligonucleotide-treated, inactivated breast cancer cells .

3. The composition of claim 2, wherein the inactivated breast cancer cells are irradiated.

4. The composition of claim 1, wherein said breast cancer cells are obtained from a patient to be treated with the therapeutic composition.

5. The composition of claim 1, wherein the immunogen is derived from a permanent breast tumor cell line.

6. The composition of claim 1, wherein the IGF-1 receptor antisense oligonucleotide comprises about 14 to about 25 sequential nucleotides.

7. The composition of claim 1, wherein the IGF-1 receptor antisense oligonucleotide has a sequence complementary to the nucleotide sequence of codons of the human IGF-1R precursor signal sequence, complementary to the nucleotide sequence at or near an initiation site, or complementary to sequences at or near a site for ribosome complex assembly.

8. The composition of claim 1, wherein the IGF-1 receptor antisense oligonucleotide includes the following sequence:

5' TCC TCC GGA GCC AGA CTT 3' [SEQ ID NO:1] or
5' ACT CGT CGG CCA GAG CGA GAG 3' [SEQ ID NO:2].

9. The composition of claim 1, wherein the IGF-1R antisense oligonucleotide comprises a synthetic base or a modified backbone.

10. An anti-breast tumor vaccine comprising:

a tumor immunogen derived from a breast cancer cell treated with an IGF-1 receptor antisense oligonucleotide in a pharmaceutically acceptable carrier.

11. An IGF-1 receptor antisense oligonucleotide having the sequence: 5' ACT CGT CGG CCA GAG CGA GAG 3' [SEQ ID NO:2].

12. A method of inhibiting the growth of breast cancer cells in a patient, the method comprising:

administering to a patient an immunogen derived from breast cancer cells treated with an IGF-1 receptor antisense oligonucleotide.

13. The method of claim 12, wherein said administering is subsequent to or concurrent with tumor reduction therapy.

14. The method of claim 13, wherein said administering is subsequent to surgical debulking of tumors.

15. The method of claim 12, wherein the IGF-1 receptor antisense oligonucleotide comprises about 14 to about 25 sequential nucleotides.

16. The method of claim 12, wherein the IGF-1 receptor antisense oligonucleotide has a sequence complementary to the nucleotide sequence of codons of the human IGF-1R precursor signal sequence, complementary to the nucleotide sequence at or near an initiation site, or complementary to sequences at or near a site for ribosome complex assembly.

17. The method of claim 12, wherein the IGF-1 receptor antisense oligonucleotide includes the following sequence:

5' TCC TCC GGA GCC AGA CTT 3' [SEQ ID NO:1] or
5' ACT CGT CGG CCA GAG CGA GAG 3' [SEQ ID NO:2].

18. A method of preparing an immunogen to inhibit growth of breast cancer cells, the method comprising:

treating breast cancer cells with an IGF-1 receptor antisense oligonucleotide; and

inactivating said breast cancer cells.

FIG. 1

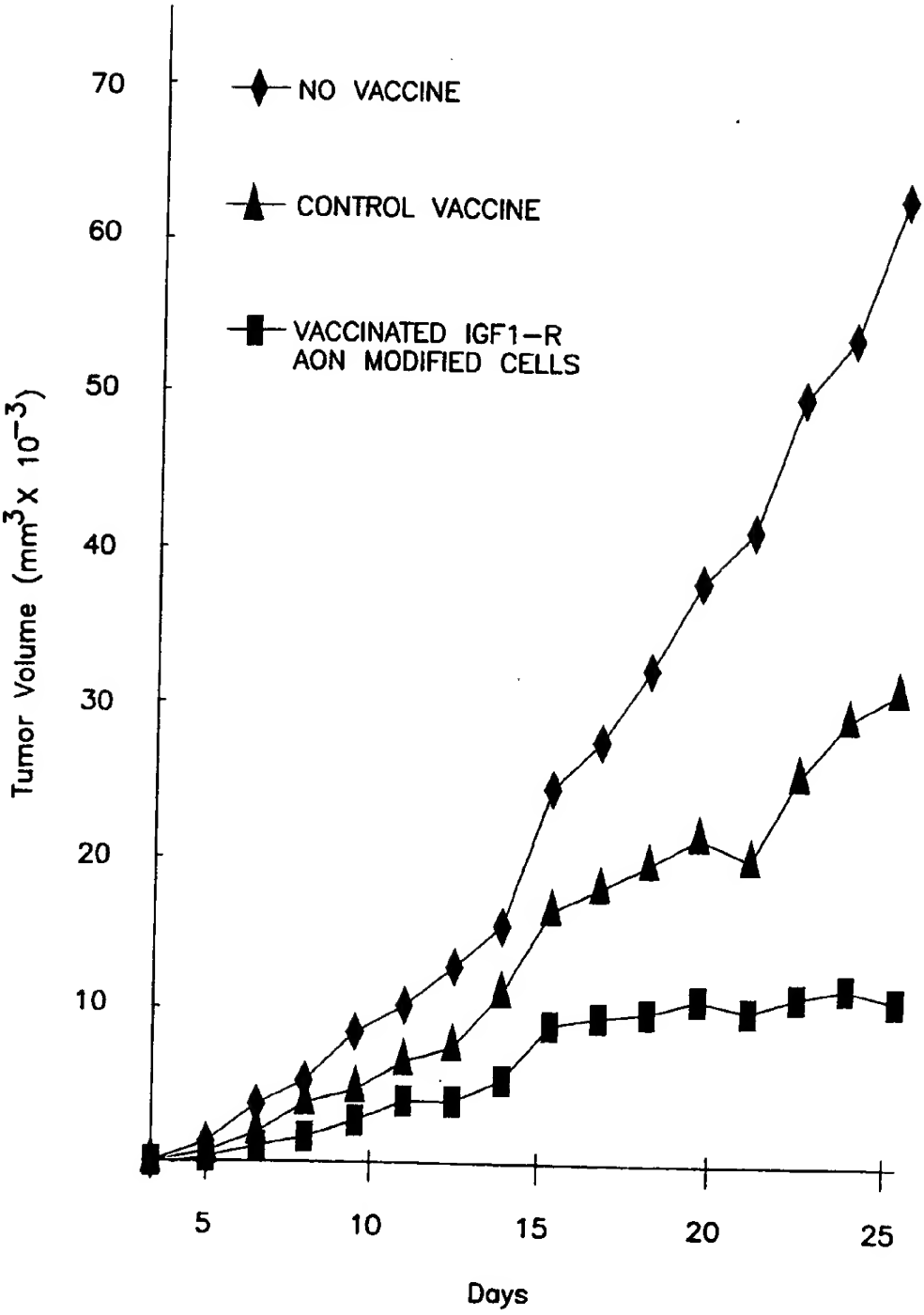
NO VACCINE
CONTROL VACCINE
VACCINATED IGF1-R AON MODIFIED CELLS
Tumor Volume ($mm^3 X 10^{-3}$)
70
60
50
40
30
20
10
5
10
15
20
25
Days

FIG. 2

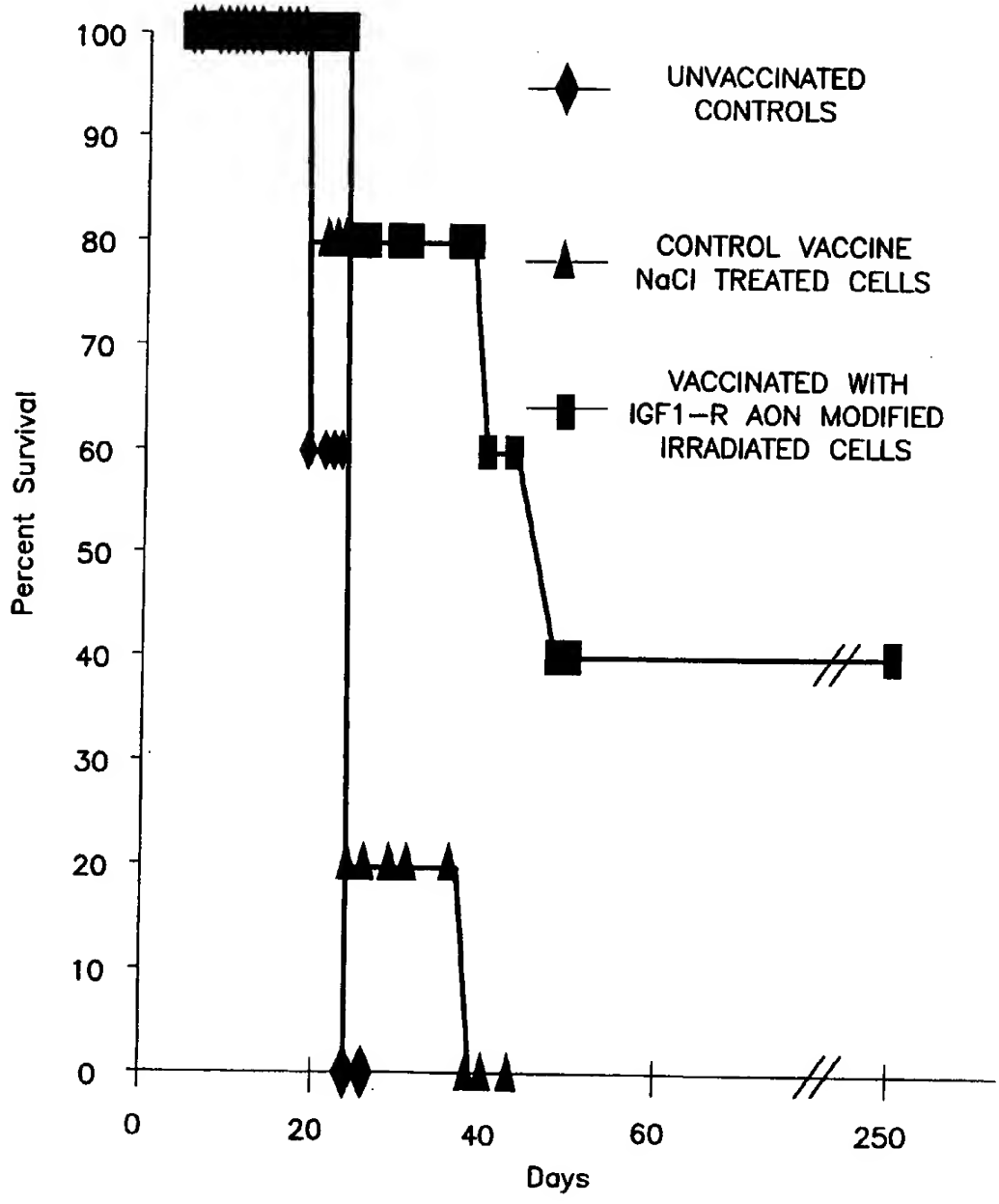
100
90
80
70
60
50
40
30
20
10
0
Percent Survival
0
20
40
60
250
Days
UNVACCINATED CONTROLS
CONTROL VACCINE NaCl TREATED CELLS
VACCINATED WITH IGF1–R AON MODIFIED IRRADIATED CELLS

FIG. 3

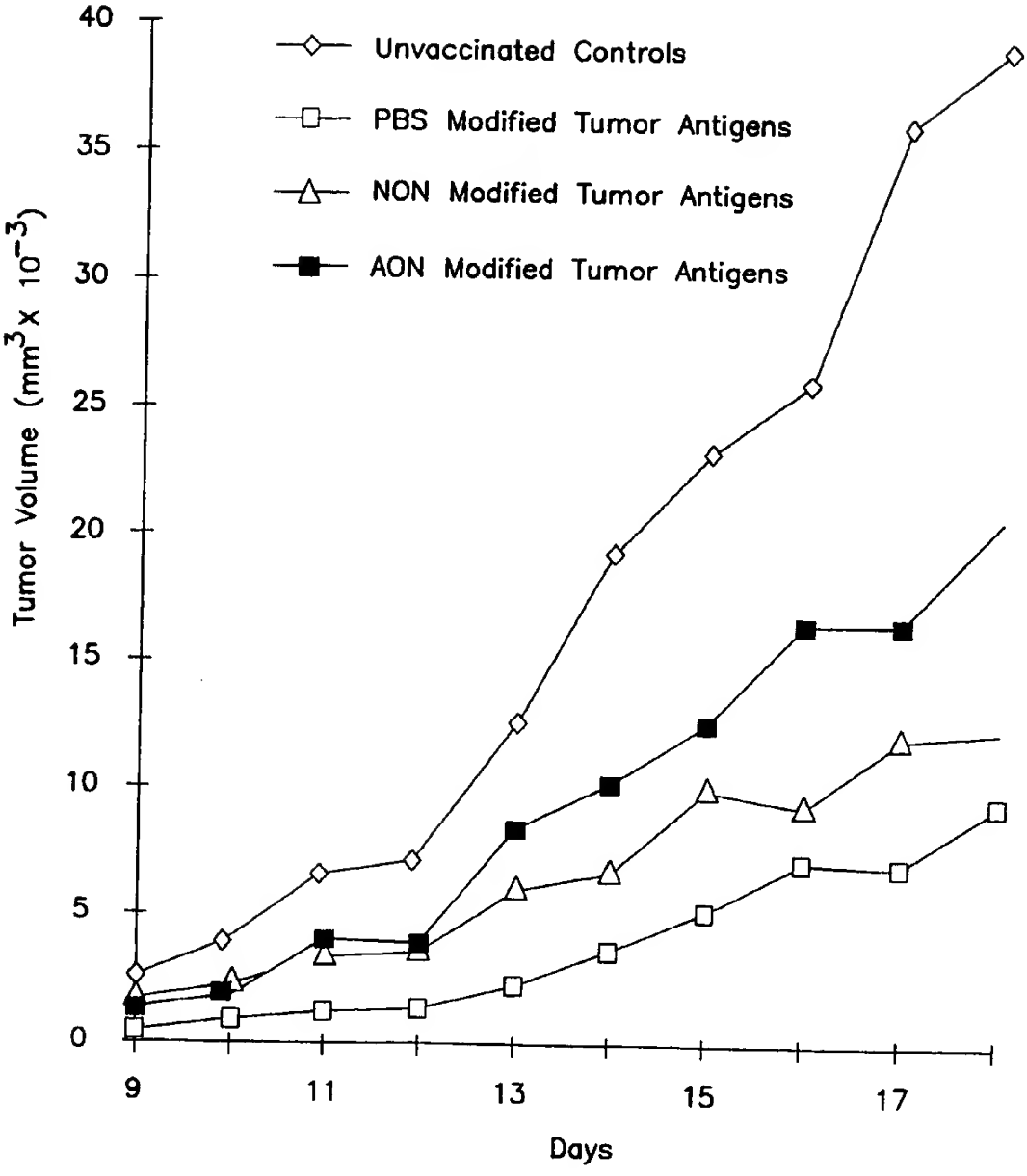
Unvaccinated Controls
PBS Modified Tumor Antigens
NON Modified Tumor Antigens
AON Modified Tumor Antigens
Tumor Volume (mm^3 X 10^{-3})
40
35
30
25
20
15
10
5
0
9
11
13
15
17
Days

FIG. 4

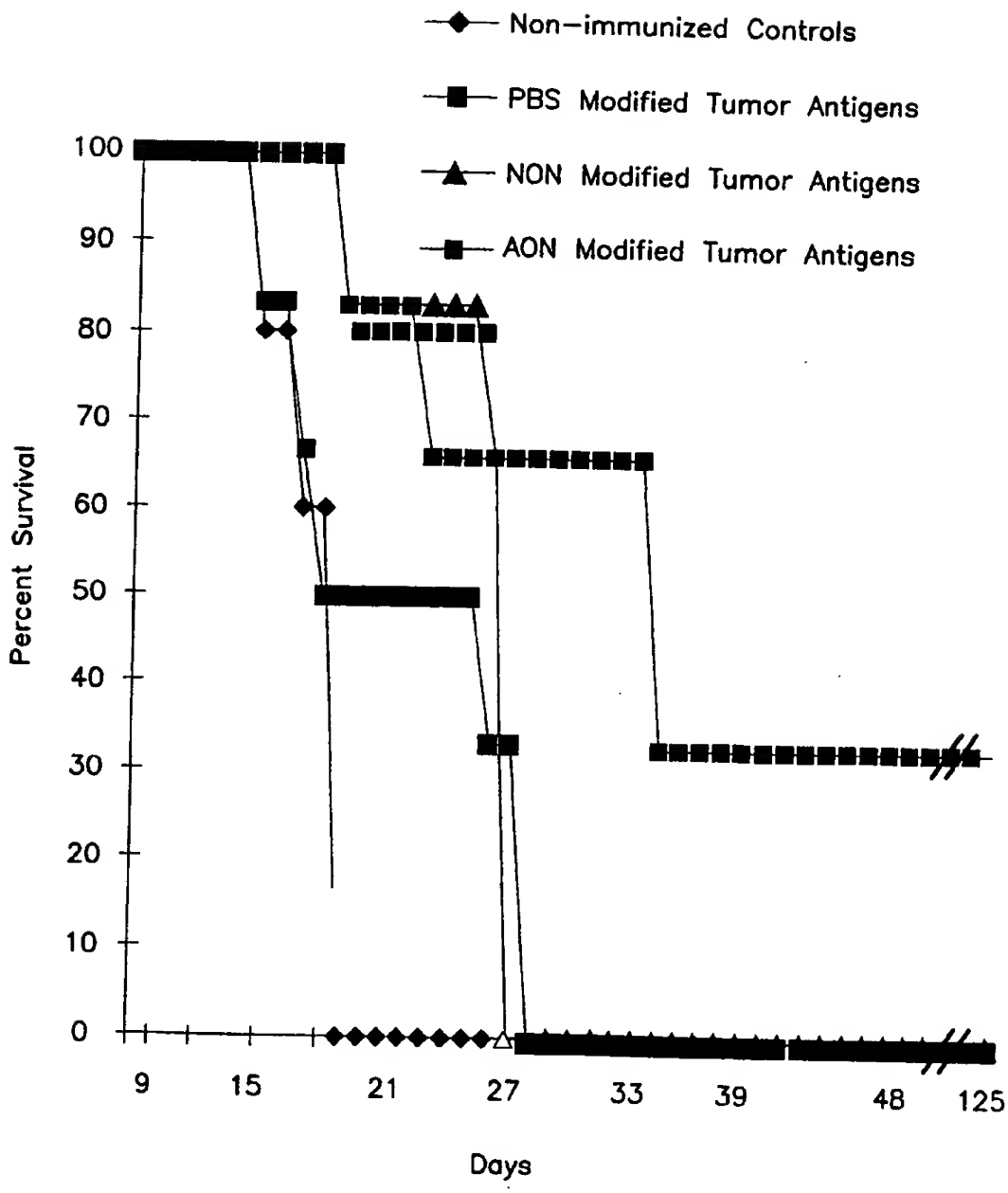
Non-immunized Controls
PBS Modified Tumor Antigens
NON Modified Tumor Antigens
AON Modified Tumor Antigens
Percent Survival
100
90
80
70
60
50
40
30
20
10
0
9
15
21
27
33
39
48
125
Days

FIG. 5

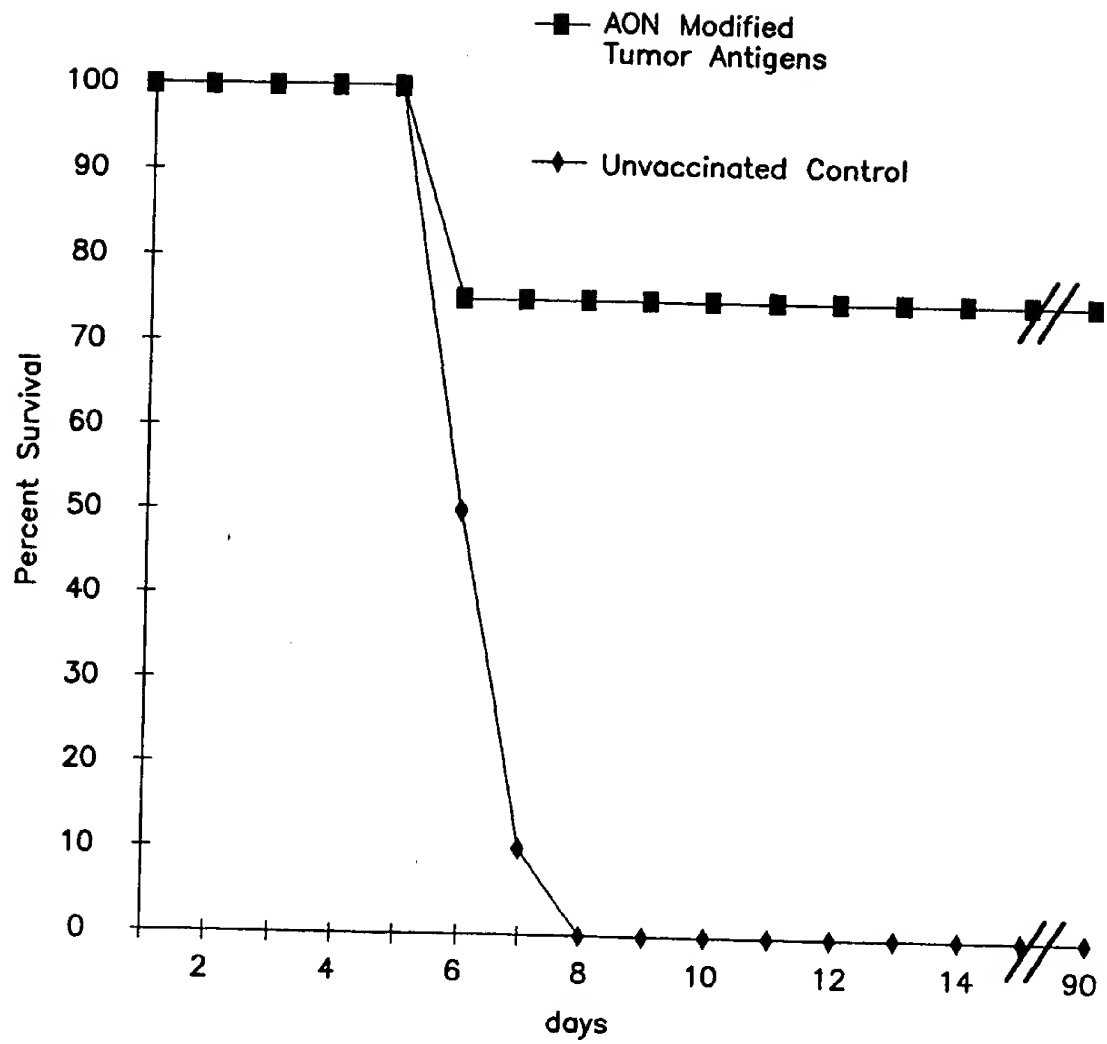
AON Modified
Tumor Antigens
Unvaccinated Control
Percent Survival
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
90
days

Fig. 6A

```
TTTTTTTTTT TTTTGAGAAA GGGAATTTCA TCCCAAATAA AAGGA ATG AAG TCT GGC   57
                                                  Met Lys Ser Gly
                                                    1

TCC GGA GGA GGG TCC CCG ACC TCG CTG TGG GGG CTC CTG TTT CTC TCC   105
Ser Gly Gly Gly Ser Pro Thr Ser Leu Trp Gly Leu Leu Phe Leu Ser
 5                  10                  15                  20

GCC GCG CTC TCG CTC TGG CCG ACG AGT GGA GAA ATC TGC GGG CCA GGC   153
Ala Ala Leu Ser Leu Trp Pro Thr Ser Gly Glu Ile Cys Gly Pro Gly
                25                  30                  35

ATC GAC ATC CGC AAC GAC TAT CAG CAG CTG AAG CGC CTG GAG AAC TGC   201
Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg Leu Glu Asn Cys
            40                  45                  50

ACG GTG ATC GAG GGC TAC CTC CAC ATC CTG CTC ATC TCC AAG GCC GAG   249
Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile Ser Lys Ala Glu
        55                  60                  65

GAC TAC CGC AGC TAC CGC TTC CCC AAG CTC ACG GTC ATT ACC GAG TAC   297
Asp Tyr Arg Ser Tyr Arg Phe Pro Lys Leu Thr Val Ile Thr Glu Tyr
    70                  75                  80

TTG CTG CTG TTC CGA GTG GCT GGC CTC GAG AGC CTC GGA GAC CTC TTC   345
Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu Gly Asp Leu Phe
85                  90                  95                  100

CCC AAC CTC ACG GTC ATC CGC GGC TGG AAA CTC TTC TAC AAC TAC GCC   393
Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe Tyr Asn Tyr Ala
                105                 110                 115

CTG GTC ATC TTC GAG ATG ACC AAT CTC AAG GAT ATT GGG CTT TAC AAC   441
Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile Gly Leu Tyr Asn
            120                 125                 130

CTG AGG AAC ATT ACT CGG GGG GCC ATC AGG ATT GAG AAA AAT GCT GAC   489
Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu Lys Asn Ala Asp
        135                 140                 145

CTC TGT TAC CTC TCC ACT GTG GAC TGG TCC CTG ATC CTG GAT GCG GTG   537
Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile Leu Asp Ala Val
    150                 155                 160
```

Fig. 6B

```
TCC AAT AAC TAC ATT GTG GGG AAT AAG CCC CCA AAG GAA TGT GGG GAC     585
Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys Glu Cys Gly Asp
165                 170                 175                 180

CTG TGT CCA GGG ACC ATG GAG GAG AAG CCG ATG TGT GAG AAG ACC ACC     633
Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys Glu Lys Thr Thr
                185                 190                 195

ATC AAC AAT GAG TAC AAC TAC CGC TGC TGG ACC ACA AAC CGC TGC CAG     681
Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr Asn Arg Cys Gln
            200                 205                 210

AAA ATG TGC CCA AGC ACG TGT GGG AAG CGG GCG TGC ACC GAG AAC AAT     729
Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys Thr Glu Asn Asn
        215                 220                 225

GAG TGC TGC CAC CCC GAG TGC CTG GGC AGC TGC AGC GCG CCT GAC AAC     777
Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser Ala Pro Asp Asn
    230                 235                 240

GAC ACG GCC TGT GTA GCT TGC CGC CAC TAC TAC TAT GCC GGT GTC TGT     825
Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr Ala Gly Val Cys
245                 250                 255                 260

GTG CCT GCC TGC CCG CCC AAC ACC TAC AGG TTT GAG GGC TGG CGC TGT     873
Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu Gly Trp Arg Cys
                265                 270                 275

GTG GAC CGT GAC TTC TGC GCC AAC ATC CTC AGC GCC GAG AGC AGC GAC     921
Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala Glu Ser Ser Asp
            280                 285                 290

TCC GAG GGG TTT GTG ATC CAC GAC GGC GAG TGC ATG CAG GAG TGC CCC     969
Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met Gln Glu Cys Pro
        295                 300                 305

TCG GGC TTC ATC CGC AAC GGC AGC CAG AGC ATG TAC TGC ATC CCT TGT    1017
Ser Gly Phe Ile Arg Asn Gly Ser Gln Ser Met Tyr Cys Ile Pro Cys
    310                 315                 320

GAA GGT CCT TGC CCG AAG GTC TGT GAG GAA GAA AAG AAA ACA AAG ACC    1065
Glu Gly Pro Cys Pro Lys Val Cys Glu Glu Glu Lys Lys Thr Lys Thr
325                 330                 335                 340
```

Fig. 6C

```
ATT GAT TCT GTT ACT TCT GCT CAG ATG CTC CAA GGA TGC ACC ATC TTC      1113
Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly Cys Thr Ile Phe
                345                 350                 355

AAG GGC AAT TTG CTC ATT AAC ATC CGA CGG GGG AAT AAC ATT GCT TCA      1161
Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn Asn Ile Ala Ser
            360                 365                 370

GAG CTG GAG AAC TTC ATG GGG CTC ATC GAG GTG GTG ACG GGC TAC GTG      1209
Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val Thr Gly Tyr Val
        375                 380                 385

AAG ATC CGC CAT TCT CAT GCC TTG GTC TCC TTG TCC TTC CTA AAA AAC      1257
Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser Phe Leu Lys Asn
    390                 395                 400

CTT CGC CTC ATC CTA GGA GAG GAG CAG CTA GAA GGG AAT TAC TCC TTC      1305
Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly Asn Tyr Ser Phe
405                 410                 415                 420

TAC GTC CTC GAC AAC CAG AAC TTG CAG CAA CTG TGG GAC TGG GAC CAC      1353
Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp Asp Trp Asp His
                425                 430                 435

CGC AAC CTG ACC ATC AAA GCA GGG AAA ATG TAC TTT GCT TTC AAT CCC      1401
Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe Ala Phe Asn Pro
            440                 445                 450

AAA TTA TGT GTT TCC GAA ATT TAC CGC ATG GAG GAA GTG ACG GGG ACT      1449
Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu Val Thr Gly Thr
        455                 460                 465

AAA GGG CGC CAA AGC AAA GGG GAC ATA AAC ACC AGG AAC AAC GGG GAG      1497
Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg Asn Asn Gly Glu
    470                 475                 480

AGA GCC TCC TGT GAA AGT GAC GTC CTG CAT TTC ACC TCC ACC ACC ACG      1545
Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe Thr Ser Thr Thr Thr
485                 490                 495                 500

TCG AAG AAT CGC ATC ATC ATA ACC TGG CAC CGG TAC CGG CCC CCT GAC      1593
Ser Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr Arg Pro Pro Asp
                505                 510                 515
```

Fig. 6D

```
TAC AGG GAT CTC ATC AGC TTC ACC GTT TAC TAC AAG GAA GCA CCC TTT    1641
Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys Glu Ala Pro Phe
            520                 525                 530

AAG AAT GTC ACA GAG TAT GAT GGG CAG GAT GCC TGC GGC TCC AAC AGC    1689
Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala Cys Gly Ser Asn Ser
        535                 540                 545

TGG AAC ATG GTG GAC GTG GAC CTC CCG CCC AAC AAG GAC GTG GAG CCC    1737
Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys Asp Val Glu Pro
    550                 555                 560

GGC ATC TTA CTA CAT GGG CTG AAG CCC TGG ACT CAG TAC GCC GTT TAC    1785
Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln Tyr Ala Val Tyr
565                 570                 575                 580

GTC AAG GCT GTG ACC CTC ACC ATG GTG GAG AAC GAC CAT ATC CGT GGG    1833
Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp His Ile Arg Gly
                585                 590                 595

GCC AAG AGT GAG ATC TTG TAC ATT CGC ACC AAT GCT TCA GTT CCT TCC    1881
Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala Ser Val Pro Ser
            600                 605                 610

ATT CCC TTG GAC GTT CTT TCA GCA TCG AAC TCC TCT TCT CAG TTA ATC    1929
Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser Ser Gln Leu Ile
        615                 620                 625

GTG AAG TGG AAC CCT CCC TCT CTG CCC AAC GGC AAC CTG AGT TAC TAC    1977
Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn Leu Ser Tyr Tyr
    630                 635                 640

ATT GTG CGC TGG CAG CGG CAG CCT CAG GAC GGC TAC CTT TAC CGG CAC    2025
Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr Leu Tyr Arg His
645                 650                 655                 660

AAT TAC TGC TCC AAA GAC AAA ATC CCC ATC AGG AAG TAT GCC GAC GGC    2073
Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys Tyr Ala Asp Gly
                665                 670                 675

ACC ATC GAC ATT GAG GAG GTC ACA GAG AAC CCC AAG ACT GAG GTG TGT    2121
Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys Thr Glu Val Cys
            680                 685                 690
```

Fig. 6E

```
GGT GGG GAG AAA GGG CCT TGC TGC GCC TGC CCC AAA ACT GAA GCC GAG    2169
Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys Thr Glu Ala Glu
        695                 700                 705

AAG CAG GCC GAG AAG GAG GAG GCT GAA TAC CGC AAA GTC TTT GAG AAT    2217
Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys Val Phe Glu Asn
    710                 715                 720

TTC CTG CAC AAC TCC ATC TTC GTG CCC AGA CCT GAA AGG AAG CGG AGA    2265
Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu Arg Lys Arg Arg
725                 730                 735                 740

GAT GTC ATG CAA GTG GCC AAC ACC ACC ATG TCC AGC CGA AGC AGG AAC    2313
Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser Arg Ser Arg Asn
                745                 750                 755

ACC ACG GCC GCA GAC ACC TAC AAC ATC ACC GAC CCG GAA GAG CTG GAG    2361
Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro Glu Glu Leu Glu
            760                 765                 770

ACA GAG TAC CCT TTC TTT GAG AGC AGA GTG GAT AAC ACG GAG AGA ACT    2409
Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn Thr Glu Arg Thr
        775                 780                 785

GTC ATT TCT AAC CTT CGG CCT TTC ACA TTG TAC CGC ATC GAT ATC CAC    2457
Val Ile Ser Asn Leu Arg Pro Phe Thr Leu Tyr Arg Ile Asp Ile His
    790                 795                 800

AGC TGC AAC CAC GAG GCT GAG AAG CTG GGC TGC AGC GCC TCC AAC TTC    2505
Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser Ala Ser Asn Phe
805                 810                 815                 820

GTC TTT GCA AGG ACT ATG CCC GCA GAA GGA GCA GAT GAC ATT CCT GGG    2553
Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp Asp Ile Pro Gly
                825                 830                 835

CCA GTG ACC TGG GAG CCA AGG CCT GAA AAC TCC ATC TTT TTA AAG TGG    2601
Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile Phe Leu Lys Trp
            840                 845                 850

CCG GAA CCT GAG AAT CCC AAT GGA TTG ATT CTA ATG TAT GAA ATA AAA    2649
Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met Tyr Glu Ile Lys
        855                 860                 865
```

Fig. 6F

```
TAC GGA TCA CAA GTT GAG GAT CAG CGA GAA TGT GTG TCC AGA CAG GAA    2697
Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val Ser Arg Gln Glu
    870                 875                 880

TAC AGG AAG TAT GGA GGG GCC AAG CTA AAC CGG CTA AAC CCG GGG AAC    2745
Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu Asn Pro Gly Asn
885                 890                 895                 900

TAC ACA GCC CGG ATT CAG GCC ACA TCT CTC TCT GGG AAT GGG TCG TGG    2793
Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly Asn Gly Ser Trp
                905                 910                 915

ACA GAT CCT GTG TTC TTC TAT GTC CAG GCC AAA ACA GGA TAT GAA AAC    2841
Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr Gly Tyr Glu Asn
            920                 925                 930

TTC ATC CAT CTG ATC ATC GCT CTG CCC GTC GCT GTC CTG TTG ATC GTG    2889
Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val Leu Leu Ile Val
        935                 940                 945

GGA GGG TTG GTG ATT ATG CTG TAC GTC TTC CAT AGA AAG AGA AAT AAC    2937
Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg Lys Arg Asn Asn
    950                 955                 960

AGC AGG CTG GGG AAT GGA GTG CTG TAT GCC TCT GTG AAC CCG GAG TAC    2985
Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val Asn Pro Glu Tyr
965                 970                 975                 980

TTC AGC GCT GCT GAT GTG TAC GTT CCT GAT GAG TGG GAG GTG GCT CGG    3033
Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp Glu Val Ala Arg
                985                 990                 995

GAG AAG ATC ACC ATG AGC CGG GAA CTT GGG CAG GGG TCG TTT GGG ATG    3081
Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly Ser Phe Gly Met
            1000                1005                1010

GTC TAT GAA GGA GTT GCC AAG GGT GTG GTG AAA GAT GAA CCT GAA ACC    3129
Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys Asp Glu Pro Glu Thr
        1015                1020                1025

AGA GTG GCC ATT AAA ACA GTG AAC GAG GCC GCA AGC ATG CGT GAG AGG    3177
Arg Val Ala Ile Lys Thr Val Asn Glu Ala Ala Ser Met Arg Glu Arg
    1030                1035                1040
```

Fig. 6G

```
ATT GAG TTT CTC AAC GAA GCT TCT GTG ATG AAG GAG TTC AAT TGT CAC    3225
Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Glu Phe Asn Cys His
1045                1050                1055                1060

CAT GTG GTG CGA TTG CTG GGT GTG GTG TCC CAA GGC CAG CCA ACA CTG    3273
His Val Val Arg Leu Leu Gly Val Val Ser Gln Gly Gln Pro Thr Leu
                1065                1070                1075

GTC ATC ATG GAA CTG ATG ACA CGG GGC GAT CTC AAA AGT TAT CTC CGG    3321
Val Ile Met Glu Leu Met Thr Arg Gly Asp Leu Lys Ser Tyr Leu Arg
          1080                1085                1090

TCT CTG AGG CCA GAA ATG GAG AAT AAT CCA GTC CTA GCA CCT CCA AGC    3369
Ser Leu Arg Pro Glu Met Glu Asn Asn Pro Val Leu Ala Pro Pro Ser
       1095                1100                1105

CTG AGC AAG ATG ATT CAG ATG GCC GGA GAG ATT GCA GAC GGC ATG GCA    3417
Leu Ser Lys Met Ile Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala
   1110                1115                1120

TAC CTC AAC GCC AAT AAG TTC GTC CAC AGA GAC CTT GCT GCC CGG AAT    3465
Tyr Leu Asn Ala Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn
1125                1130                1135                1140

TGC ATG GTA GCC GAA GAT TTC ACA GTC AAA ATC GGA GAT TTT GGT ATG    3513
Cys Met Val Ala Glu Asp Phe Thr Val Lys Ile Gly Asp Phe Gly Met
                1145                1150                1155

ACG CGA GAT ATC TAT GAG ACA GAC TAT TAC CGG AAA GGA GGC AAA GGG    3561
Thr Arg Asp Ile Tyr Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly
          1160                1165                1170

CTG CTG CCC GTG CGC TGG ATG TCT CCT GAG TCC CTC AAG GAT GGA GTC    3609
Leu Leu Pro Val Arg Trp Met Ser Pro Glu Ser Leu Lys Asp Gly Val
       1175                1180                1185

TTC ACC ACT TAC TCG GAC GTC TGG TCC TTC GGG GTC GTC CTC TGG GAG    3657
Phe Thr Thr Tyr Ser Asp Val Trp Ser Phe Gly Val Val Leu Trp Glu
   1190                1195                1200

ATC GCC ACA CTG GCC GAG CAG CCC TAC CAG GGC TTG TCC AAC GAG CAA    3705
Ile Ala Thr Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn Glu Gln
1205                1210                1215                1220
```

Fig. 6H

```
GTC CTT CGC TTC GTC ATG GAG GGC GGC CTT CTG GAC AAG CCA GAC AAC    3753
Val Leu Arg Phe Val Met Glu Gly Gly Leu Leu Asp Lys Pro Asp Asn
                1225                1230                1235

TGT CCT GAC ATG CTG TTT GAA CTG ATG CGC ATG TGC TGG CAG TAT AAC    3801
Cys Pro Asp Met Leu Phe Glu Leu Met Arg Met Cys Trp Gln Tyr Asn
            1240                1245                1250

CCC AAG ATG AGG CCT TCC TTC CTG GAG ATC ATC AGC AGC ATC AAA GAG    3849
Pro Lys Met Arg Pro Ser Phe Leu Glu Ile Ile Ser Ser Ile Lys Glu
        1255                1260                1265

GAG ATG GAG CCT GGC TTC CGG GAG GTC TCC TTC TAC TAC AGC GAG GAG    3897
Glu Met Glu Pro Gly Phe Arg Glu Val Ser Phe Tyr Tyr Ser Glu Glu
    1270                1275                1280

AAC AAG CTG CCC GAG CCG GAG GAG CTG GAC CTG GAG CCA GAG AAC ATG    3945
Asn Lys Leu Pro Glu Pro Glu Glu Leu Asp Leu Glu Pro Glu Asn Met
1285                1290                1295                1300

GAG AGC GTC CCC CTG GAC CCC TCG GCC TCC TCG TCC TCC CTG CCA CTG    3993
Glu Ser Val Pro Leu Asp Pro Ser Ala Ser Ser Ser Ser Leu Pro Leu
                1305                1310                1315

CCC GAC AGA CAC TCA GGA CAC AAG GCC GAG AAC GGC CCC GGC CCT GGG    4041
Pro Asp Arg His Ser Gly His Lys Ala Glu Asn Gly Pro Gly Pro Gly
            1320                1325                1330

GTG CTG GTC CTC CGC GCC AGC TTC GAC GAG AGA CAG CCT TAC GCC CAC    4089
Val Leu Val Leu Arg Ala Ser Phe Asp Glu Arg Gln Pro Tyr Ala His
        1335                1340                1345

ATG AAC GGG GGC CGC AAG AAC GAG CGG GCC TTG CCG CTG CCC CAG TCT    4137
Met Asn Gly Gly Arg Lys Asn Glu Arg Ala Leu Pro Leu Pro Gln Ser
    1350                1355                1360

TCG ACC TGC TGA TCCTTGGATC CTGAATCTGT GCAAACAGTA ACGTGTGCGC ACGCGC 4195
Ser Thr Cys
1365

AGCGGGGTGG GGGGGGAGAG AGAGTTTTAA CAATCCATTC ACAAGCCTCC TGTACCTCAG  4255
TGGATCTTCA GTTCTGCCCT TGCTGCCCGC GGGAGACAGC TTCTCTGCAG TAAAACACAT  4315
TTGGGATGTT CCTTTTTTCA ATATGCAAGC AGCTTTTTAT TCCCTGCCCA AACCCTTAAC  4375
TGACATGGGC CTTTAAGAAC CTTAATGACA ACACTTAATA GCAACAGAGC ACTTGAGAAC  4435
CAGTCTCCTC ACTCTGTCCC TGTCCTTCCC TGTTCTCCCT TTCTCTCTCC TCTCTGCTTC  4495
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Fig. 6I

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ATAACGGAAA AATAATTGCC ACAAGTCCAG CTGGGAAGCC CTTTTTATCA GTTTGAGGAA  4555
GTGGCTGTCC CTGTGGCCCC ATCCAACCAC TGTACACACC CGCCTGACAC CGTGGGTCAT  4615
TACAAAAAAA CACGTGGAGA TGGAAATTTT TACCTTTATC TTTCACCTTT CTAGGGACAT  4675
GAAATTTACA AAGGGCCATC GTTCATCCAA GGCTGTTACC ATTTTAACGC TGCCTAATTT  4735
TGCCAAAATC CTGAACTTTC TCCCTCATCG GCCCGGCGCT GATTCCTCGT GTCCGGAGGC  4795
ATGGGTGAGC ATGGCAGCTG GTTGCTCCAT TTGAGAGACA CGCTGGCGAC ACACTCCGTC  4855
CATCCGACTG CCCCTGCTGT GCTGCTCAGG GCCACAGGCA CACAGGTCTC ATTGCTTCTG  4915
ACTAGATTAT TATTTGGGGG AACTGGACAC AATAGGTCTT TCTCTCAGTG AAGGTGGGGA  4975
GAAGCTGAAC CGGC                                                    4989
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/20815

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K45/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07H C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WALLENFRIEDMAN M A ET AL: "IGF - 1 R antisense and nonsense oligonucleotide therapy for 9L glioblastoma results in an immune response against an unrelated syngeneic tumor in Fischer 344 rats." 26TH ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, WASHINGTON, D.C., USA, NOVEMBER 16-21, 1996. SOCIETY FOR NEUROSCIENCE ABSTRACTS 22 (1-3). 1996. 948. ISSN: 0190-5295, XP002055143 see the whole document --- -/--	1-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 1998	20. 03. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mennessier, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/20815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WALLENFRIEDMAN M A ET AL: "Effects of IGF-1R antisense and nonsense oligonucleotide administration on 9L glioblastoma and MAT B3 breast cancer tumor growth in Fischer 344 rats." 87TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, WASHINGTON, D.C., USA, APRIL 20-24, 1996. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 37 (0). 1996. 354. ISSN: 0197-016X, XP002055144 see the whole document ---	1-18
A	BASERGA R: "Controlling IGF-receptor function: a possible strategy for tumor therapy" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 5, May 1996, page 150-152 XP004035784 see the whole document ---	1-18
A	WO 94 22486 A (UNIV JEFFERSON ;BASERGA RENATO (US); SELL CHRISTIAN (US); RUBIN RA) 13 October 1994 see page 33; figure 7A ---	8,11,17
P,X	WALLENFRIEDMAN M ET AL: "Peripheral vaccine for breast cancer: Insulin-like growth factor receptor (IGF - 1R) antisense oligonucleotide (AON) modified tumor cells protect against peripheral disease and brain metastases." EIGHTY-EIGHTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, SAN DIEGO, CALIFORNIA, USA, APRIL 12-16, 1997. PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING 38 (0). 1997. 617. ISSN: 0197-016X, XP002055145 see the whole document -----	1-18

4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/ 20815

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 12 - 17
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 12-17 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/20815

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9422486 A	13-10-94	CA 2158347 A	13-10-94
		EP 0750516 A	02-01-97
		JP 8508405 T	10-09-96
		US 5643788 A	01-07-97